

## IN THE SPECIFICATION:

Please replace the paragraph beginning on page 30, line 2 with the following:

The following reagents were used for polynucleotide (PNK) reactions: an oligonucleotide substrate; T4 PNK (~~New England Biolabs~~) (NEW ENGLAND BIOLABS™);  $\gamma$ -<sup>32</sup>P-ATP, 6000 Ci/mmol (Amersham); the buffer: 70 mM Tris pH 7.6, 10 mM MgCl<sub>2</sub>, and 5 mM DTT; TE (10 mM Tris pH 8.0, 1 mM EDTA); and, distilled, deionized water.

Please replace the paragraph beginning on page 30, line 6 with the following:

The following reagents were used for primer extension reactions: a PAGE purified Substrate oligonucleotide with a 3' Binding Domain; PAGE purified Template Nucleic Acid; 10x stock buffer (100 mM Tris pH 7.5; 50 mM Mg Cl<sub>2</sub> 75 mM DTT); a stop solution (10 mM EDTA); 10  $\mu$ M dATP (unlabeled); and exo<sup>-</sup> Klenow DNA polymerase (~~5 units/  $\mu$ l, New England Biolabs~~ (5 units/  $\mu$ l, NEW ENGLAND BIOLABS™).

Please replace the paragraph beginning on page 30, line 11 with the following:

Equipment and supplies included: 500  $\mu$ l microfuge tubes; 1.5 ml screw cap microfuge tubes; ~~Sephadex~~ SEPHADEX™ (G25 spin columns); a waterbath set to 94°C; a waterbath set to 37°C; a tabletop microcentrifuge; and a micropipette with aerosol barrier tips.

Please replace the paragraph beginning on page 30, line 32 with the following:

The Substrate Nucleic Acid was resuspended in TE at a final concentration of 0.5  $\mu$ M (0.5 picomoles/ $\mu$ l. From this stock, 2 picomoles (4  $\mu$ l) was 5' end labeled using T4 PNK. The Substrate Nucleic Acid was incubated with 100  $\mu$ Ci  $\gamma$ -<sup>32</sup>P-ATP and 5 units of T4 PNK in a final reaction volume of 50  $\mu$ l with kinase buffer of composition as described above for 1 hour at 37°C. Unincorporated  $\gamma$ -<sup>32</sup>P-ATP was removed by passage through a ~~Sephadex~~ SEPHADEX™ G-25 spin column. Radiolabel incorporation was measured using scintigraphy.

Please replace the paragraph beginning on page 33, line 36 with the following:

From the 10  $\mu$ M stock of Substrate Nucleic Acid, 2 picomoles (2  $\mu$ l) was 5'-end labeled using T4 PNK, as described in Example 1 (Section 6.2, *supra*). Briefly, Substrate Nucleic Acid was incubated with 100  $\mu$ Ci  $\gamma$ - $^{32}$ P-ATP and 5 units of T4 PNK in a final reaction volume of 50  $\mu$ l with kinase buffer of composition as described for 1 hour at 37  $^{\circ}$ C. Unincorporated  $\gamma$ - $^{32}$ P-ATP was removed by passage through a ~~Sephadex~~ SEPHADEX<sup>TM</sup> G-25 spin column. Radiolabel incorporation was measured using scintigraphy and, assuming no loss of labeled nucleic acid or contamination with unincorporated label, resulted in a specific activity of  $1 \times 10^7$  CPM per picomole probe. Structure of this labeled probe is shown in FIG. 4A.

Please replace the paragraph beginning on page 34, line 12 with the following:

The annealing step of the primer extension reaction was carried out as described in Example 1 (Section 6.3, *supra*). Briefly, 1  $\mu$ l (0.5 picomoles) of Substrate Nucleic Acid (from the dilute "working solution"), 1  $\mu$ l of 10x reaction buffer, and 1  $\mu$ l (12.5 picomoles) of Template Nucleic Acid were added together in a 500  $\mu$ l microcentrifuge tube in the same molar concentrations as shown in Table 2. After heating the sample to 94 $^{\circ}$ C (1 minute) and cooling to room temperature (5 minutes), 6  $\mu$ l of  $\alpha$ - $^{32}$ PdATP and 1  $\mu$ l (5 units) of the exo Klenow fragment of *E. coli* DNA polymerase were added. The reaction was allowed to proceed at room temperature for one hour. 40  $\mu$ l of stop solution (10 mM EDTA) was added and the reaction was passed through a ~~Sephadex~~ SEPHADEX<sup>TM</sup> G25 spin column to remove unincorporated  $\alpha$ - $^{32}$ PdATP. An aliquot was removed and evaluated for radiolabel incorporation by liquid scintillation counting. Assuming no loss of labeled nucleic acid or contamination with unincorporated label, the probe had a specific activity of  $9 \times 10^7$  CPM per picomole, nearly 10x that of the probe labeled with PNK. The structure of the labeled probe produced is shown in FIG. 4A.

Please replace the paragraph beginning on page 36, line 11 with the following:

From the 10  $\mu$ M stock of Substrate Nucleic Acid, 2 picomoles (2  $\mu$ l) was 5'-end labeled using T4 polynucleotide kinase, as described in Example 2 (Section 7.2, *supra*). Substrate Nucleic Acid was incubated with 100  $\mu$ Ci  $\gamma$ - $^{32}$ P-ATP and 5 units of T4 PNK in a final reaction volume of 50  $\mu$ l with kinase buffer for 1 hour at 37  $^{\circ}$ C. Unincorporated  $\gamma$ - $^{32}$ P-ATP was removed by passage through a ~~Sephadex~~ SEPHADEX<sup>TM</sup> G-25 spin column.

Radiolabel incorporation was measured using scintigraphy and, assuming no loss of labeled nucleic acid or contamination with unincorporated label, resulted in a specific activity of  $8 \times 10^6$  CPM per picomole probe. Structure of this labeled probe is shown in FIG. 5A.

Please replace the paragraph beginning on page 36, line 21 with the following:

The annealing step of the primer extension reaction was carried out as described previously. Briefly, 1  $\mu$ l (0.5 picomoles) of Substrate Nucleic Acid (0.5  $\mu$ M stock), 1  $\mu$ l of 10X reaction buffer, and 1  $\mu$ l (12.5 picomoles) of Template Nucleic Acid were added together in a 500  $\mu$ l microcentrifuge tube. After heating the sample to 94 °C (1 minute) and 25 cooling to room temperature (5 minutes), 6  $\mu$ l of  $\alpha^{32}$ P dATP and 1  $\mu$ l (5 units) of the exo Klenow fragment of *E. coli* DNA polymerase were added. The reaction was allowed to proceed at room temperature for one hour. 40  $\mu$ l of stop solution (10 mM EDTA) was added and the reaction was passed through a **Sephadex** SEPHADEX™ G25 spin column to remove unincorporated  $\alpha^{32}$ P-dATP. An aliquot was removed and evaluated for activity by liquid 30 scintillation counting. Assuming no loss of labeled nucleic acid or contamination with unincorporated label, the probe had a specific activity of  $7 \times 10^7$  CPM per picomole, about 10 times that of the probe labeled with PNK. The structure of the labeled probe produced is shown in FIG. 5A.

Please replace the paragraph beginning on page 36, line 37 with the following:

To directly compare the functional sensitivity of a PNK 5' end-labeled probe and a 3' primer-extension labeled probe using the method of the invention, two identical Northern blots were prepared using human placental RNA. Ten micrograms of total RNA (Ambion**AMBION**™) was separated on a formaldehyde-agarose gel (Current Protocols in Molecular Biology, John Wiley & Sons, Inc.). After electrophoresis, RNA was transferred to MSI nylon membranes (Micron Separations, Inc.) by capillary action in 20x SSC and baked at 80°C for 1 hour. The membranes were prehybridized in PerfectHyb™ (Sigma) at 60°C for one hour. Hybridization was carried out for 4 hours at 60°C in 10 mls total volume of PerfectHyb™ with  $10^7$  CPM of probe added (final  $10^6$  CPM/ml). Probes were hybridized to separate blots in parallel. Blots were washed twice in 5x SSC (0.75 M NaCl, 0.075 M

NaCitrate) at 55°C for 30 minutes. Blots were exposed to a Packard MP Phosphor Screen for one hour and visualized using a Packard Cyclone™ Storage Phosphor System.

Please replace the paragraph beginning on page 38, line 3 with the following:

The Substrate Nucleic Acids were resuspended in TE at a final concentration of 0.5  $\mu$ M (0.5 picomoles/  $\mu$ l). From this stock, 2 picomoles (4  $\mu$ l) was 5' end labeled using T4 PNK. Substrate Nucleic Acids were each incubated with 100  $\mu$ Ci  $\gamma$ -<sup>32</sup>P-ATP and 5 units of T4 PNK in a final reaction volume of 50  $\mu$ l with kinase buffer for 1 hour at 37 °C. Unincorporated  $\gamma$ -<sup>32</sup>P-ATP was removed by passage through a Sephadex™ SEPHADEX™ G-25 spin column. Radiolabel incorporation was measured using scintigraphy.